This Thesis Has Been

MICROFILMED

Negative No. T. 665

Form 26
Anatomy of Ascidia viridis n.sp.

by

Homer Allin Hill

Missouri State University
May 1902
Anatomy of Ascidia viridis

All the observations for this description were made from specimens preserved either in formalin or picric formalin. The formalin specimens, which were kept in the test, were found to be in the best condition. Those preserved in picric formalin were more or less contracted.

The material was collected by Dr. George Shelfer in the harbor of Beaufort, N. C. during the summer of 1901. It was dredged up in water from three to six fathoms deep, and was found in great abundance. Frequently the individuals adhere in small clumps, but are easily separated.

In every case where examination was made the branchial sac was found to harbor a small amphipod (species undetermined).
Diagnosis of species
Body attached, sessile
Branchioph and atrial aperture
not close together; each & broad
(counted with microscope, test removed)
Test rather tough
Mantle delicate on left side,
musculature on right side and
over siphons.
Branchioph sac not pleated;
not folded on itself postibly
Internal longitudinal bars papillate,
stigmata straight.
Nerve ganglins and mesal gland
distant from dorsal tubercle;
Dorsal lamina a simple
membrane, transversely rifted.
Edge slightly curved; it
is continued behind the
Oesophageal aperture
Teratacles simple, numerous.
(about 40)
Alimentary Canal on left side
of branchial sac
Sends in the intestinal loop's
This form in shape is not unlike that of Ascidianema, which is given by Herdman as the shaft of a typical Ascidia. The body is oblong, flattened laterally, the posterior end is rounded, the anterior end is rather flat and slightly narrowed (Fig. 1). It is attached, often to the inside of a fragment of different shell, by the posterior left side.

The branchial aperture is circular, terminating and median. The atrial aperture is elliptical, on the dorsal border almost half way between the anterior and posterior ends; it is directed slightly posteriorly. There are no distinct lobes around either aperture. Though the surface is raised somewhat, and irregular, in these regions, the entire surface is smooth but marked by irregular and

Herdman, W. A., 1882, Report on the Tunicata collected during the voyage of H. M. S. Challenger during the years 1873 to 1876, Part I, Ascidia, ascidiae.
Unequal primums and elevations.
The color is pale, whitish yellow.
The length of the body is 10 cm., the breadth 4 cm., the thickness 3 cm.
The test varies in thickness from 2 to 5 mm.; it is a little thicker on the left side than on the right; the thickest portions are around the aperatures, and the thinnest over the place of attachment to the shell.
It is gelatinous, almost cartilaginous, but translucent as steel in life, the loops of the intestine may be distinctly seen through the test.
Blood vessels may be clearly distinguished from the interior.
Two blood vessels from the body proper enter the test at a point just beneath the atrial siphon and unite low on the left side. From there they branch out in all directions gradually becoming smaller and terminating in coarse capillaries, each of which shows a terminal ampulla (Fig 2). The ends of the main branches thus terminate, and on the right side
A cross section of the test shows a matrix with closely set bladder cells, and a few pigment cells. (Fig 2). Objects, such as small sand granules, annelid worms, and sand particles, are found imbedded in the test. A large portion of the surface is also covered by a brown, hydrated mucus somewhat imbedded.

On splitting open the test, the body proper is easily removed. It is attached very loosely by the blood vessels, and at the siphons. The general shape is that of the outside of the test (Fig. 3a & b). The drawing shows the siphons considerably contracted, due to the effect of the preserving fluid. The length of the body after it has been removed is 1.3 cm., the breadth is 2.5 cm. and the thickness 1 cm. These dimensions and those given above apply to the larger specimens.

The most prominent feature of the right side is the muscular tube. (Fig. 3-6)
The mantle on this side and over the siphons (extending back to the intestinal loop first) the oral siphon, on left side) is composed of a network of muscles. Without any apparent arrangement except over the siphons where they seem to be arranged quite systematically into longitudinal and circular muscles.

The circular muscles are nearer the exterior than the longitudinal. (Fig. 485)

Each muscle of the mantle is made up of a bundle of very fine fibrillae, quite loosely enclosed in a sheath of sarcoplasm. (Fig. 6)

No muscular structure has been observed. In none of the sections in which muscles were cut transversely did the fibrillae appear circular. (Fig. 18, 20, 21). A vertical section of the mantle (Figs 20, 21) shows that the majority of the muscles are situated under the inner edge, and that the outer part is composed of connective tissue.
With blood spaces in every part and bounded by the thyroid. Each aperture has eight small and unequal lobes.

The oral siphon is lined by a thin layer of test extending down to the testicular ring. The tentacles are situated at the opening into the branched chamber at the base of the oral siphon, they are placed in one very slightly bent row, support muscular ring, and extend into the cavity but appear to be able to bend back and forth through the opening. They are simple, practically of one size, about one hundred and forty in number (Fig. 7).

The atrial siphon is slightly shorter than the oral and extends a little to the right of the median line.

In Herdman's Revised Classification of the Tunicata, the author gives a note in which he makes mention of Ascidia demersa, from the Malay Archipelago, which has ten loses on each aperture. (Described by Sluiter.)
At its base it is provided with quite closely arranged, strong circular muscles. The circular muscles converge toward the orifice. On the inner surface of this orifice are found scattered papillae, short, becoming longer in region of sphincter, with the point directed toward the aperture. (Fig. 3) There are more these papillae in outer half of orifice. Many of these, as well as the tentacles in oral siphon, are filled with blood corpuscles (Fig. 2).

The atrial cavity is a comparatively thin, open space nearly the same size as the large branchial sac. The branchial cavity extends throughout the entire length and breadth of the body. The branchial wall is attached on the left side by septa and blood vessels, extending across the atrial cavity, to the viscera. On the right side quite strong septa connect it in the same way with the mouth.
The structure of the branched wall is complex. In the innermost plane are found the internal longitudinal bars (Fig. 8) having quite regular papillae on one side, just opposite each papilla is a short duct which connects its longitudinal bar with the transverse vessel. The transverse vessels, in a plane more external, are not of exactly equal size, but there is evidently no regularity about the arrangement of the large and small vessels. In a third and still more external plane are found some of the finer longitudinal vessels and a part of the stigmata, from three to five appearing in each mesh. The stigmata are not uniform either in size or shape; they are ciliated (Fig. 8) and lined with branched epithelial cells. The rest of the finer longitudinal vessels and the remainder of the stigmata are arranged in rows perpendicular to the rows shown in Fig. 9. The wall is thus quite thick, and
The cut ends of all fibrous longitudinal vessels in each mesh appear in cross section, to form a quadrangle with one end open (the outer surface of wall) (Fig. 10). This section cuts across end of the transverse vessels at a very oblique angle. At a point between the ends of the transversely arranged rows of stigmata which gives the appearance of one large vessel.

A similar section in another place would show also the external transverse vessels (Fig. 11).

A fourth place from the inner bend quite near the outer surface shows a network of blood vessels (Fig. 11). b and c in Fig. 10 and 11 are the corresponding spaces between the vessels. Between each of the external transverse vessels is a short transverse vessel connecting the two rows of fibres.
longitudinal vessels which border adjacent meshes. (Fig. 11. Ep. 10) shows how the section passed through one of these short connecting vessels. There is a slight variation in the branchial wall in different regions. Sometimes three or four of these short transverse vessels occur. In every case they extend beyond the fund longitudinal vessels which the connect, somewhat resembling paccal.

One of the cut septa which connect the branchial wall with the renatha may be seen in Fig. 10. (Ep.)

The position of the endostyle is typical. It connects anteriorly with the peribranchial groove (Fig. 12) which extends around the oral siphon, and unite again to form the epibranchial groove in the dorsal region. This quite a deep groove with edges free and flaring.
It extends ventrally around the posterior end of the branchial sac, then turned dorsally to the oesophageal aperture. It may be seen in the wall of the oesophagus from the exterior (test removed).

The endostyle in cross-section shows a thick lining of epithelial pad, which reaches nearly to the free edge of the sides (Fig. 13). This epithelial pad, on each side of the groove, is thrown into short transverse folds (Figs. 12-8, 13). Long flagella arise from the bottom of the groove. Short cilia are found on the sides for a short distance, about half way down and over the free edges. Branchial epithelium begins where the epithelial pad leaves off and continues over the outside to the point of union.

This condition is possibly artificial as it was observed, however, in both the formalin and para-
formaldehyde material.
With the branchial wall and then continues ventrally around the endostyle (Fig. 13). The ventral edge of the endostyle forms the upper wall of the atrial cavity in this region.

From the extremity posterior, median, point to the osophagus the endostyle is considerably modified; the epithelial pad is wanting; there are no long flagellae. The sides are covered with short, without cilia, while the very bottom is lined with short cilia (Fig. 14).

The dorsal lamina is a narrow membrane, extending from the posterior end of the epi-branchial groove to the posterior end of the branchial sac. It becomes narrower back of the osophagial aperture. The lamina and endostyle meet at its point where the endostyle becomes modified. The left edge of the endostyle continues forward as the lamina; the right edge
Continues forward as the right and longer side of the modified endostyle.

On the other (left) side of the modified endostyle there is a division between these two at the point of divergence.

Near the base of the dorsal lamina is a short fold, which, with the lamina as one side, forms a more or less irregular groove, or canal. These two grooves continue forward to the oesophagus, parallel to each other, each one forming the boundary of a large dorsal groove (Fig. 14). This dorsal groove is lined with a slightly modified branchial epithelium. Short cilia are found on one side of the fold near base of lamina and extending out from this a short distance (Fig. 14).

Continuing to the oesophagus, at the lamina is broader, but the dorsal groove is not so wide and it is not bounded on either side by a smaller groove.
A few short cilia are found near base of lamina and also on one side of a small fold at the distal edge from lamina of dorsal lamina to not a perfectly straight membrane but is curved to the right.

The transverse ribs are on the left, or convex side. The posterior portion of the rib does not extend quite half way to the free edge but a much rounded fold or rib does continue (Fig. 14, 8, 16).

The free edge is not plain but slightly crenate.

The heart is similar to the heart of other Aesodida. It is a long circular tube, extending on the ventral edge, from a point at the end with the end of the anterior loop of the intestine, back around the posterior end of the body to a position near with the dorsal end of the stomach.

Most of the heart is visible only on the left side. A smaller portion may be seen only on the right side (Fig. 3, 28b).
The wall presents an irregular spiral or folded appearance. Although it is not so, yet similar in appearance to the heart of Polypoda angustifolius described by William and Poor.

Outside of the heart is a light colored spherical mass (sometimes two) which moves freely from one end to the other. Apparently forced along by the pulsations. The nature of this body was not determined.

In general the blood vessels are quite well defined tubes. Members of these vessels are cut across in removing the branches from the vessels of the left side. Two of the largest vessels in the body lead from a point near the anterior end of the heart out into the test (Fig. 3, a). A central vessel is


(page 37) (Issued Sept 28 1872)
Situated beneath the endostyle (Fig. 13) and a partially defined dorsal vessel above the lamina and dorsal groove (Fig. 14 & 15) there are, however, blood spaces in every part of the body where the blood may flow freely without being confined to definite channels. Such blood spaces are in the mantle (Figs. 18, 20, 21) in the wall of the stomach and intestines (Figs. 26 & 27); throughout the subumbrellar stomach, the interumbrellal organs (Fig. 8, 22) and among the glands of the reproductive organs throughout all these spaces and in the course of all the blood vessels among innumerable blood corpuscles. These corpuscles are light green and, in life, in account of their great numbers, they give the body a decided green color. It is possible that they are not blood corpuscles at all, but a kind of green alga, living symbiotically.
in the blood. They appear singly
and collected in groups of six
or more (Figs 14, 15, 20 & 21). Many of them show nuclei.
In others no nucleus can be
made out. They are round,
but not all of equal size.
Under the high power of
immersion each one appears to be a Colony.
Probably they represent a
form of protozooids.

Dorsal tubercles, the
epibranchial groove, the new
ganglion and visual gland
are all present (Fig. 17).

The distance from the posterior
end of the epibranchial groove
to the anterior end of the ganglion
is not quite equal to the
distance from the same point
to the dorsal tubercle: which
latter distance is the length of

One objection to considering them
protozooids is the fact that
after the material has been
sectioned and mounted, with staining
and dehydrating process, the green color
persists.
The epibranchial groove.

The dorsal tubercle is situated near the tentacular ring and at the place where the peripharyngeal groove enters the epibranchial groove is shifted a little to the right of a line drawn longitudinally through the groove (Fig. 17). The endings of the tubercle have an epithelial lining and are ciliated (Fig. 18). The character of the epithelium and the length of the cilia vary in different regions. The duct of the neural gland leads directly into the tubercle dorsally. It is embedded in the mouth near the lower surface below the anterior nerves (Fig. 18, 21). It has an epithelial lining throughout its entire length.

The ganglion is situated dorsally about the sixth of the distance between the siphoes.

The relative position of the ganglion and neural gland is somewhat...
similar to that in Asthenotyla. The gland lies beneath the ganglion and projects beyond it at each end and on each side (Fig. 19). But it is asymmetrically situated. The ganglion does not lie over the median line of the gland. At the anterior end the gland projects on the left side and not on the right, while at the posterior end the condition is just reversed. The larger mass of the gland is on the right side.

A transverse section (Fig. 20) through the anterior end of ganglion shows the gland medially on the left side, extending only to the median longitudinal line.
A similar section through the posterior end would show the same relation, but reversed position.

Blood sinuses completely surround the gland and ganglion. They are also included by the atrial epithelium which lines the mouth (Fig. 20).

The large uterus of the neural gland is situated close to the ventral side of the ganglion, part of it lying beneath the anterior duct and also extending back beyond the posterior duct (Fig. 21). The figure does not show a median section. Blood vessels of the bursa and wall appear beneath and at the side of the gland (Fig. 20).

The duct of the neural gland is continuous with this lumen extending directly beneath the base of the mouth.

I have not been able to demonstrate a separate duct posteriorly, either by longitudinal or transverse sections.

The nerve ganglia is elongated.
With its long axis in the long axis of the body (Fig. 19), it is quite firmly attached to the muscles by connective tissue. From nerves are given off anteriorly and posteriorly; these do not originate in the same transverse planes at either end. The ganglion lies further anteriorly on the left, and posteriorly on the right side (Fig. 19). The same general description is true for the neural gland with regard to its shape. Fig. 22 shows the relative position of the anterior nerves, duct and gland. The different size of the nerves in this section may be understood by an inspection of Fig. 19.

The epibranchial groove is dilated (Fig. 18). Its inner surface is not regular but is thrown into longitudinal folds, which are apparently continuations of the pericycral bands.
The digestive tract is visible only on the left side. The intestine is large and makes a double loop, which occupies all of the space dorso-ventrally, and nearly all the space antero-posteriorly, having the shape of an enlarged S turned backward.

The oesophagus and arms are placed dorsally. The arms open into the atrial cavity near the base of the atrial siphon.

The oesophagus is situated back of the atrial siphon, a little to the left of the median line about 2/3 of the distance from the anterior to the posterior end of the body. The oesophageal aperture opens out of the branchial cavity and becomes much smaller just outside of the branchial wall.

The dorsal groove, described above, is in direct communication with the funnel shaped oesophageal opening. After this constriction, the
Aesophagus gradually widens again as it enters the stomach (Fig. 2. a).

The stomach is an elongated, circular sac, situated in the posterior part on the left side of the body, only the heart being more posterior (Fig. 3. a). The long axis is placed dorso-ventrally with either end constricted dorsally into the aesophagus. Ventrally into the intestine. The posterior loop of the intestine lies over the anterior edge of the stomach. Thus, giving the stomach from the anterior an crescent shaped appearance.

The inner surface of the stomach is thrown into longitudinal folds. These folds are large on the right side. The folds just described converge into the end of the esophagus where it arises at the beginning of the intestine and continues throughout its entire length. It is placed ventrally except in the loops.
of the intestine. While it is situated
slightly up and closer to the wall
on the inside of the loop.
It stops just back of the anal
opening. No specialized epithelial
lining of the stomach or intestine
can be demonstrated.

The anal opening is plain
and is not covered by disassociated
epithelium (Fig. 24).

In all of the observed specimens
the stomach and intestine were
filled with foetal matter.

Reproductive Organs

The reproductive organs are easily distinguished in the
loops of the intestine (Fig. 34)
the larger space is in the anterior
loop. By sectioning and
examination with the microscope
the anterior loop is found to
contain ovaries. The posterior
loop being filled with testes.

Also, the gonads are imbedded
in the wall of the intestine which
is made up of rather coarse
fibrovascular and connective tissue
(Fig. 25). Male gonads are
also found in the wall of the
Stomach (Fig. 26)

In the wall of the intestine between the loops, both male and female gonads are found together. (Fig 24)

The ova develop in all stages a layer of follicle cells around each ovum which clearly shows the nucleus and nucleolus. (Fig 24).

The male lobules are filled with seminiferous tubules (Figs 24 & 26).

The spermatozoa, seen in a cross section, of the testes are very small, elongated, and provided with a minute short flagella.

The oviduct arises from a network of small tubes which ramify among the ovaries, and follows the posterior loop of the intestine around to the anus. (Fig 39).

The vesicula seminalis is the right wall of the intestine at about this point when the oviduct first becomes visible. The ves. deferens then
follows the course of the conduct, but a little below and slightly in front of it. This may be seen after careful inspection.

These termini, at the same place near the end of arms and near fluted spines (Fig 24). In several individuals observed the was defenceless filled with spermatozoa and the nidus filled with eggs. This effect both maturing at the same time.

The wall of the stomach is filled with esophageal bodies. Its seminal vesicles containing convolutions, these are more abundant in the right wall and over the end of the typhloskele. In the region named, after the stomach has been stained in brown Carmine, these vesicles are so numerous and so closely set that they render the wall quite opaque.

Dr. Ætene demonstrated, in bringing that self-fertilization is possible in this animal. And embryos thus produced were raised into larvae.

The propagation of eggs fertilized in this way is not as great, however, as in cross-fertilization, and more abnormalities occur.
In the left wall of the stomach they appear in great numbers only as more scattered (Fig. 27). They appear also throughout the entire length of the intestine even among the glands wherever these occur (Fig. 23).

I have made only a cursory examination of the coagulants. Evidently the treatment given the sections containing them was not adapted to the bringing out of the details of their further structure.

Their general appearance is that of a large round vesicle with a darker stained body enclosed. Frequently more than one coagulant may be seen within a single vesicle. The surface of each vesicle shows polygonal cells of pavement epithelium (Fig. 28). Fig. 26 shows a characteristic appearance of the renal convoluted vesicle in section.
The general structure is that of concentric rings which, in some cases, lie so close together as to leave no space between them, as seen in the figure. They do not all show a double center.

**Systematic Position**

**Class:** Tunicata
**Order:** Ascidiae
**Suborder:** Ascidiae Inflatae
**Family:** Ascididae
**Subfamily:** Ascidinidae
**Genus:** Ascidia
**Species:** A. viniidi

**Methods.** For Tolu preparations, the material was stained in bismarck carmine. The sections were stained with safranin.

For Fig. 19 a slight differentiation of nerve and muscle was effected by staining with bismarck carmine. Thorough washing and afterward staining with 2.5% eosin and washing out in water.
Explanation of Plates

All of the figures excepting 1, 3, 5, 8, 20 are outlined with the camera lucida.

Plate I

Fig. 1. Whole Specimen, Ascidioiinds (photograph)

Fig. 2. Cross section of test. X 525

Fig. 3. Body removed from test. Natural size. A, left side; B, right side

Fig. 4. Portion of wall of oral siphon. Seen from interior, with test lining removed. X 50

Fig. 5. Portion of atrial siphon, seen from the interior. X 85

Fig. 6. Broken end of muscle from mantle. X 85

Fig. 7. Pharyngeal opening and inter-bipodial organs. The oral siphon, above the tentacle, has been removed. The tentacles, ring then cut and spread out with the tentacles directed outward X 10 (seen from the interior)
Fig. 8. Portion of branchial wall seen from inside X 8.5
Fig. 9. Small portion of branchial wall (tangential section) X 52.5
Fig. 10. Transverse section branchial wall X 70
Fig. 11. Tangential section of same near outer wall X 8.5

Plate II

Fig. 12. Union of endostyle and peripharyngeal groove X 50
Fig. 13. Cross section endostyle X 8.5
Fig. 14. Cross section, dorsal region of branchial sac, back of esophagopal opening X 8.5
Fig. 15. Cross section of same anterior to odopharyngeal opening X 8.5

Fig. 16. Dorsal lamina X 70
Fig. 17. Dorsal tubercle, inversion X 70

Fig. 18. Longitudinal vertical section of dorsal tubercle, showing cut side of epibranchial groove with folds (continuation of peripharyngeal bands) X 70
Fig. 19. Heron ganglion and renal gland. Setae from entering branchial wall (part of the muscles removed) X 50

Plate III

Fig. 20. Transverse section of ganglion and gland. Showing also thickness of its wall X 70

Fig. 21. Longitudinal, vertical section of ganglion and gland. The section is not a median section X 70

Fig. 22. Section same as Fig. 20, really more anterior. Details not filled in) X 70

Fig. 23. Cross section of wall of stomach. Showing folds on right side (flattened surface) X 70

Fig. 24. Areas also the end of oviduct & vas deferens X 50

Fig. 25. Portion of cross section wall of intestines, showing male & female glands in trabecular of connective tissue X 50
Fig 26. Cross section upper wall of stomach showing muscle, serosa, & submucosa.

Fig 27. Upper wall of stomach (stomach polyp) seen from interior. X 15

(*the blood vessels & nerve vessels*)
Reference Letters

a = annis  
a.ep. = atrial epithelium  
a.m. = ampullar blood vessel in Test  
a.s. = atrial siphon  
b.c. = blood capusel  
b.ep. = branchial epithelium  
b.s. = blood sinus  
b.v. = blood vessel  
c.d. = Connecting duct  
c.i.m. = circular muscle of siphon  
d. = duct of neural gland  
d.b.s. = dorsal blood sinus  
d.d. = dorsal lamine  
d.t. = dorsal tuberly  
et. = ectoderm  
e.g. = epibranchial groove  
e.m. = endostyle  
e.p.p. = epithelial pad  
eg.t.v. = external transverse vessel branchial  
f. = fiber of muscle bundle  
f.l.v. = fine longitudinal vessel branchial  
fl. = flagella  
fg. = nerve ganglion  
glf. = neural gland  
ht. = heart  
i.l. = internal longitudinal bar of branchial wall
Reference Letter (continued)

int. = intestine
l.m. = longitudinal muscle
lu. = lumen of neural gland
mus. = muscle
nu. = nerve
O. = ovary
or. = oesophagus
o.s. = oral siphon
ov. = oviduct
p. = papilla
p. e. = pigment cell
p.p.g. = peripherayal groove
r.c. = neural coordination
s. = stigma of branch of wall
sep. = septum
st. = stomach
t. = testis or testes
ten. = transverse muscle
tn. = tentacle
t.r. = transverse int. = dorsal lamina
t.v. = transversely vessel of branchial wall
v.d. = vas deferens
vs. = vessel of neural coordination
v.v. = ventral vessel (blood)
Synopsis of Contents

Collection of preserving material ........................................ 1
Diagnosis of the species .................................................. 2
General description .......................................................... 3
Test .................................................................................. 4
Describing body (testicular) .................................................. 5
   a. Presentation ............................................................... 5
   b. Specimen ................................................................. 7
   c. Counts ................................................................... 8
Branchial Sac ........................................................................ 9
   a. Endostyle ................................................................ 10
   b. Dorsal Sinus ............................................................. 13
Heart .............................................................................. 15
Blood Circulation .............................................................. 18
Intesinalized Organs .......................................................... 18
   a. Dorsal tube .............................................................. 19
   b. Vein and gauflin ......................................................... 19
   c. Renal gland .............................................................. 20
Digestive System ............................................................... 23
Reproductive Organs ......................................................... 24
Renal System ..................................................................... 27
Classification .................................................................... 29
Methods ........................................................................... 29
Explanation of plates ......................................................... 30
Reference sections ............................................................. 34
This thesis is never to leave this room. Neither is it to be checked out overnight.